Studies on the Degradation Behavior of Chitosan-g-Poly (acrylic acid) Copolymers

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Abstract

Graft copolymers of Chitosan and poly(acrylic acid) were synthesized with a redox initiator, cerium ammonium nitrate. After 2 h of reaction at 70 ^{o}C, a gel product was obtained. The swelling ratio of the copolymer in water depends on the pH value and has a maximum in the buffer solution at pH 7. This is because the morphological structure of the copolymers in the water changes with the pH values. In addition, the swelling ratio of the copolymer increases with the feeding amount of acrylic acid monomer. The degradation behavior of the copolymers was observed both in a lysozyme solution and an active slurry solution. The degradation rate was higher in the lysozyme solution and depended on the composition of the copolymers.

***Key Words*:** Chitosan, Poly(acrylic acid), Graft Copolymer, Degradation, Lysozyme, Slurry

1. Introduction

Hybridization of natural polymers with synthetic polymers is of great interest because of their application to biomedical and biodegradable materials [1]. One of the natural polymers that have attracted great attention recently is chitosan. Chitosan is a high-molecular-weight polysaccharide composed mainly of β-(1,4) linked D-glucosamine and partially of β-(1,4) linked N-acetyl-D-glucosamine. It is generally prepared by the partial deacetylation of chitin in a hot alkali solution. Chitin is the most abundant natural polymer next to the cellulose and can be found in the skeletal materials of crustaceans and insects, and cell walls of bacteria and fungi. Chitin and chitosan can be used in the fields of wastewater treatment, food processing, cosmetics, pharmaceuticals, biomaterials and agriculture [2,3]. With its fibrous structure, chitin is hardly soluble in any solvent. Yet, chitosan can be dissolved in an acid solution and becomes a cationic polymer because of the protonation of amino groups on the C-2 position of pyranose ring.

In this study, cerium ammonium nitrate (CAN) was used to initiate the graft copolymerization of acrylic acid (AA) onto chitosan. Hopefully, new material with desired properties can be achieved by the chemical combination of natural and synthetic polymers. PAA has been used in the adhesives and super-absorbent polymers, because of the pendant carboxyl groups. Chitosan is a material with several important advantages including biocompatibility, biodegradability and anti-bacterial. Both of them can absorb a great amount of water. Therefore, chemical combination of chitosan and PAA might have potential in the use of the super-absorbent material with anti-bacterial and biodegradable properties. The water absorption and degradability behavior, were

thus examined in this article.

2. Experimental

2.1 Materials

Chitosan was obtained from Tokyo Chemicals Inc., Tokyo. The degree of deacetylation was found to be 86% by a colloid titration method [4], and the viscosity average molecular weight was found to be 616,000 by a viscometric method, where the Mark-Houwink constants k and α were 1.38 x 10^{-2} and 0.85, respectively [5]. AA monomer from Tedia Chemical Company was distilled under reduced pressure. Only the distillate obtained at the middle stage of distillation was used for polymerization. CAN, ($Ce(NH_4)_2(NO_3)_6$), was a reagent-grade from Showa Chemical Inc. All the other chemicals were analytical-grade or above and used as received without further purification. Lysozyme was obtained from Merck (Germany) and has an activity of 100,000 U/mg. The active slurry was obtained from a local bakery company and its bacterial density was 2.4 x 10^7 CFU/mL.

2.2 Synthesis of Graft Copolymer

A specific amount of chitosan was first dissolved in the aqueous solution of acrylic acid. The solution was purged with nitrogen and heated to 70 ^oC in an isothermal water bath. The cerium ammonium nitrate was dissolved in 20 mL of water and pre-heated to 70 ^oC, before it was poured into the solution. After 2 hours of reaction, chitosan-*g*-PAA copolymer was formed and the solution became a gel. The gel solution was taken out for dialysis to remove the residual ceric ion and all other small molecules. To prepare the sample membranes for the test of swelling and degradation properties, the gel was put into a stainless mold and then dried for 48 h in a circulation oven followed by another 48 h in a vacuum oven at 60^oC. The reaction conditions and the sample codes of membranes are listed in Table 1. In addition, the conversion was calculated with the following equation.

$$X\,(\%) = \frac{W_1 - W_{CS}}{W_{AA}} \times 100 \qquad (1)$$

where W_{CS} and W_{AA} are the initial weight of chitosan and AA monomer, respectively, and W_1 is the final weight of drying product.

Table 1. Reaction conditions and the sample codes of various membranes

System	CAC510	CAC520	CAC540
CS (g)	5	5	5
AA (g)	10	20	40
CAN (mole)	0.01	0.01	0.01
H_2O (g)	500	500	500
Temperature (^oC)	70	70	70

2.3 Swelling Studies

Samples were immersed into several buffer solutions with different pH values at 30 ^oC to observe the swelling behavior. The swollen samples were removed at various time intervals, and the excess water was removed from the sample surface with filter paper. The weight of swollen samples was measured, and the samples re-immersed into the buffer solution. The procedure was repeated until there was no further weight increase. The swelling ratio was calculated by the weight of swollen sample at a specific time divided by the initial weight in the dry state.

2.4 Degradation Behaviors

Thermal degradation behavior was investigated with a method of thermal gravimetric analysis (TGA-7 from Perkin-Elmer). The weight loss of sample was measured in a temperature range of 30 to 550 ^oC with a heating rate of 10 ^oC/min. To investigate the enzymatic degradation of the copolymer sample, a buffer solution containing 0.025 M Na_2HPO_4/KH_2PO_4 was prepared by adjusting the pH value to 7 with a 0.1 M HCl solution at 30 ^oC. Approximately 0.1-g sample membrane was put into a 30 mL of the above buffer solution. After the addition of 0.01 g of lysozyme, the sample membrane was taken out at several interval times, rinsed with the deionized water repeatedly and then dried. The weight loss was measured to evaluate the degradation. In another experiment to investigate the degradation behavior in active slurry, 0.1-g sample membrane was put into a beaker with a 30-mL of TSB solution, followed by the addition of 5 mL of the active slurry solution. The beaker purged with air was set in an isothermal shaker at 100 rpm and 25 ^oC. After a certain period of time, the sample was taken out, rinsed, dried and weighed.

3. Results and Discussion

3.1 Synthesis of the Copolymer

Ceric ion (Ce^{4+}) is a strong redox initiator, which

can oxidize the pyranose ring of polysaccharide resulting in the formation of a free radical on the ring [6-10]. Chitosan-g-PAA thus can be obtained by the grafting of acrylic acid monomer onto the chitosan following the traditional free radical polymerization procedure. In addition, poly(acrylic acid) homo-polymer is also formed during the polymerization, through the chain transfer to monomer. The conversion of acrylic acid monomer after 2 h of reaction increased from 80 % to 91 %, as the monomer feed increased from 10 g to 40 g based on 5 g of chitosan (Figure 1). The increase in conversion was due to the decrease in solution viscosity when more acrylic acid was added. In all the cases, a gel was formed after the reaction. A structure similar to the cross-linked network is proposed for the reaction product, Figure 2. When chitosan was dissolved in the acrylic acid solution, some of the amino groups became positive-charged due to the reaction with the proton dissociated from the acrylic acid, $NH_2 + H^+ \rightarrow NH_3^+$. At the same time, the grafted PAA became negative-charged from the carboxylate groups, $COOH \rightarrow COO^- + H^+$. As a result, both chitosan and grafted PAA chains were extended and more rigid because of the repulsive force from the same charges along the chains. However, when the extended PAA chains intersect with the chitosan chains, ionic force between the negative-charged carboxylate group and the positive-charged amino group could hold them together and served as a cross-linking point. Since both of them are hydrophilic polymers, they can absorb a lot of water and lock these water molecules in this pseudo cross-linked structure.

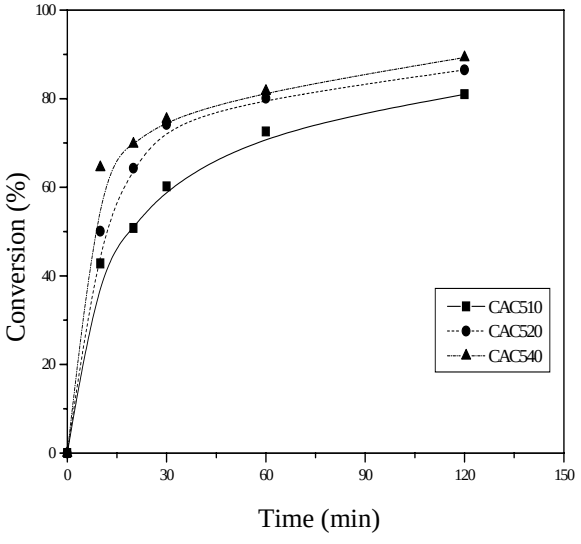

Figure 1. The effect of AA monomer amount on the conversion as function of reaction time

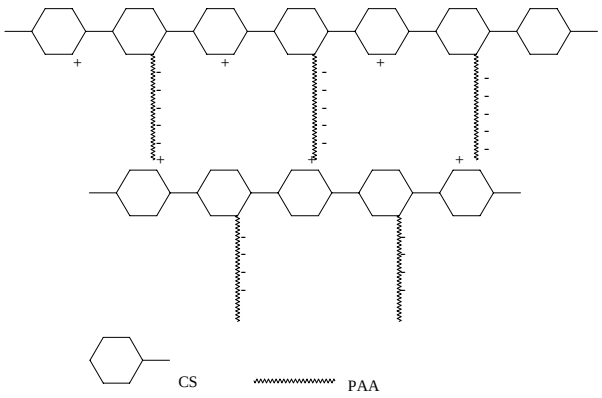

Figure 2. Proposed structure for the copolymer gel

3.2 Swelling Behavior

Figure 3 shows the swelling behavior of the sample membranes (2-mm thickness) in several buffer solutions with different pH values. The swelling ratio depends on pH values and the amount of monomer feed to the reaction system. For the pure chitosan, the swelling ratio increased as pH value decreased. This is because in a more acidic solution, more amino groups become protonated. At pH 4, 95% of amino groups exist as positive-charged NH_3^+ [11]. These positive-charged groups along the chitosan chains exert repulsive force, which consequently extend the chitosan chains. Therefore, it can absorb more water; since the structure loosen up. In addition, the charges in the chitosan chains result in an increase in the ion concentration and thus the osmotic pressure inside the structure, which makes the water easily to diffuse into the structure due to the thermodynamic driving force. Because of these reasons, the swelling ratio is higher in the acidic solution.

For the copolymer samples, the swelling behavior is also dependent on the structure of the sample, which changes with pH value. It is mentioned previously that the reaction product became a gel with a lot of water inside the structure and a pseudo cross-linked structure was proposed. However, in the buffer solution at pH 4, the dissociation of carboxylic acid in PAA is suppressed, where only 20% of the carboxylic acid dissociate [12,13]. As a result, most of the PAA chains are not as rigid as that in pH 7 solution and also fewer cross-linking points is expected. Therefore, the swelling ratio was lower than that in the neutral solution. At high pH value as in the basic solution, chitosan chains no longer bear charges, though most carboxyl groups in PAA dissociate. The pseudo cross-linked structure again collapses. Therefore, the copolymer sample at pH

7 had the higher swelling ratio than those at pH 4 or pH 9. In addition, the swelling ratio increased with the AA monomer feed, which reached 32 when the monomer feed is 40 g based on 5 g of chitosan.

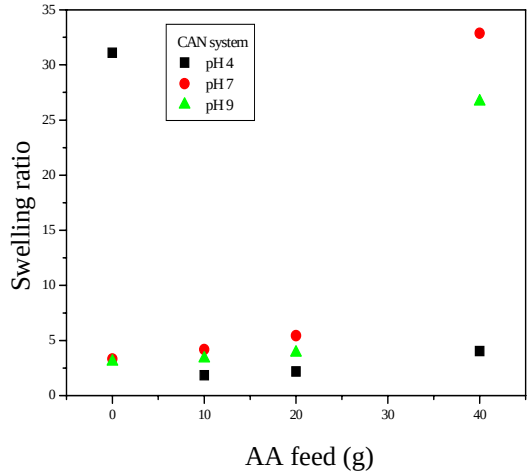

Figure 3. The effect of pH value on the swelling ratio of sample membrane as function of AA feed

3.3 Thermal Degradation Behavior

The thermal degradation behavior of pure chitosan and PAA are shown in Figure 4. Two-stage degradation behavior was observed for PAA. The first stage, in the range of 250 to 350 ^{o}C, was probably caused by the dehydration of carboxylic acid and decarboxylation. The second stage mainly after 350 ^{o}C was due to the chain scission in the main chain. Chitosan started to degrade at 250 ^{o}C and had a broad degradation temperature range with a high char yield at 550 ^{o}C. The degradation mechanism is very complex including the dehydration, deacetylation and chain scission. For the copolymer samples, the ceric ion initiator had to be removed by a dialysis method before running any thermal degradation studies. Otherwise, it would oxidize chitosan and PAA at high temperatures. The thermal degradation curves of various copolymer samples are shown in Figure 5, where three stages were observed. The first-stage degradation from 200 to 250 ^{o}C was attributed to the loss of water due to the amidation from the carboxylate group in PAA with the associated positive-charged amino group in chitosan. This amidation was evidenced by some other techniques such as Infrared spectro-photometer, which are not shown here [14]. Both the deacetylation of chitosan and the dehydration as well as the decarboxylation of PAA chains caused the second-stage degradation from 250 to 350 ^{o}C. The third-stage degradation behavior from 350 to 525 ^{o}C was mainly due to the chain scission both in PAA and chitosan. It was found that the higher the monomer feed, the lower the char yield at 550 ^{o}C. This is because the char yield of pure PAA is much lower than that of pure chitosan.

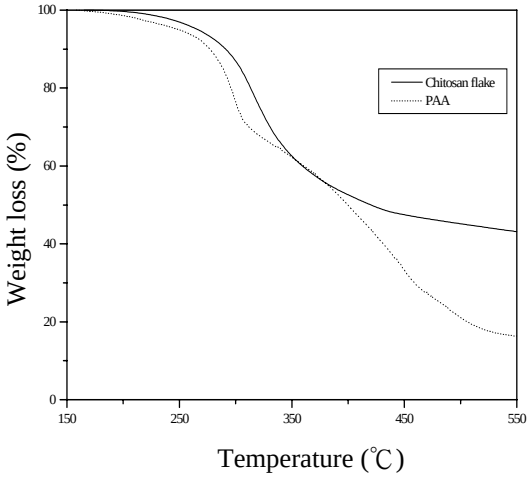

Figure 4. The weight loss of pure chitosan and PAA as function of temperature

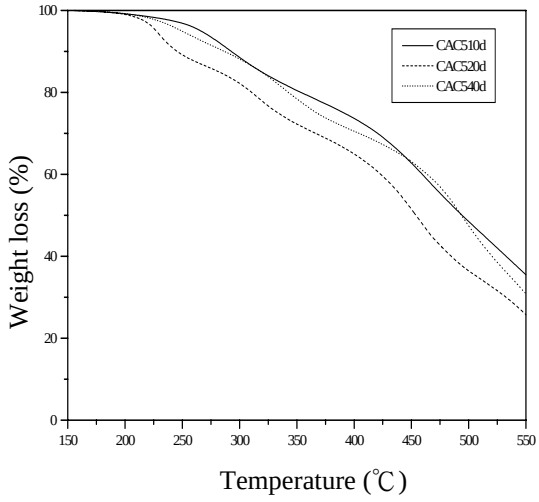

Figure 5. The weight loss of several sample membranes as function of temperature

3.4 Enzymatic Degradation

Lysozyme can interact with chitosan and facilitate the hydrolysis of chitosan, especially in an acid solution at pH 5-6. Therefore, lysozyme was used to evaluate the enzymatic degradation of the copolymer samples and the results are shown in Figure 6. The weight loss increased with the degradation time for a CAC510 sample membrane until it reached a plateau value, which

was about one third of the initial weight after 4 days. Yet, the rate was slower at the first two days, and then increased in the following two days. After that, no more degradation occurred, indicating that the remaining was not degradable by the lysozyme. In the same Figure, it was found that the weight loss of the CAC510 was only slightly smaller than the pure chitosan. It is assumed that the weight loss was mainly caused or at least initiated from the degradation of chitosan, since the experimental result shows that pure PAA does not degrade by the lysozyme. If the weight loss was re-calculated based only on the chitosan component in the copolymer (the weight loss based on the whole sample divided by the weight content of the chitosan component, Table 2), a value of 85% was obtained for the CAC510 sample. This means that 85% of the chitosan component degraded away, which is much higher than the pure chitosan. If this were true, a conclusion would be reached that the degradation rate of chitosan was higher in the copolymer than the pure form, which is not necessary true. Further investigation from the FTIR and elemental analysis of the dissolved fragments in the solution, the evidence of PAA chain was actually found. Therefore, it is more plausible to assume that some of the grafted PAA chains, especially the short ones, are easily dissolved into the water accompanied with the degraded chitosan fragments. This would increase the weight loss of the copolymer sample. In addition, if the monomer feed was increased, chance of forming short grafted PAA chains was less. Therefore, the weight loss for these systems should be smaller as can be seen in Table 2, if one compares CAC510, CAC520 and CAC540 samples.

There are many microorganisms in the active slurry and some of them can contact and adhere to the surface of the sample membranes. The enzymes secreted from these micro-organisms may degrade the copolymer samples and, the degradation fragments can be served as the nutrition or carbon source for the micro-organisms. Figure 7 shows the weight loss of the copolymer samples in an active slurry solution increased with the degradation time. The weight loss was higher for the copolymer samples than the pure chitosan under the same conditions. However, compared with the results in the directly enzymatic degradation, the degradation rate was slower. This is because the former was the direct use of the enzyme to degrade the sample, and the latter was the secreted enzymes by the microorganisms.

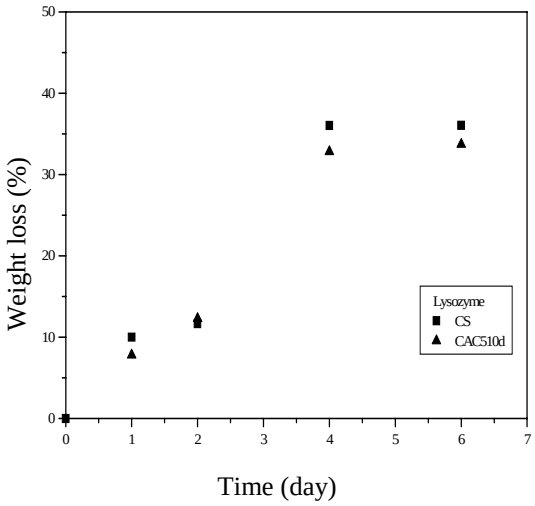

Figure 6. The weight loss of pure chitosan CS and a sample CAC510 under enzymatic degradation of lysozyme as function of time

Table 2. Weight loss of various samples at 48 and 96 h of lysozyme degradation

Sample	CAC510	CAC520	CAC540
CS content in the copolymer	38.2 %	22.4 %	12.2 %
Weight loss (%) at 48 h	12.3 %	4.7 %	3.6 %
Weight loss (%) at 96 h	32.8 %	9.8 %	6.1 %

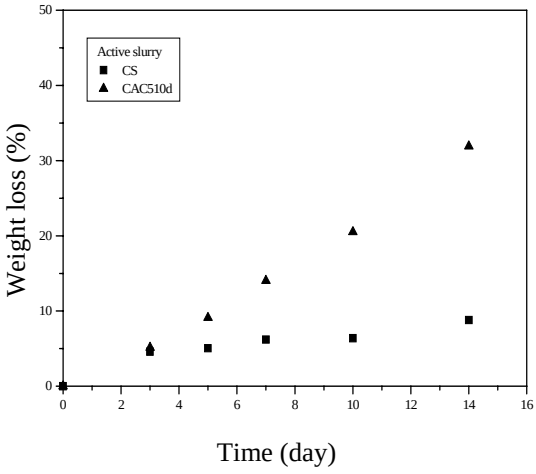

Figure 7. The weight loss of pure chitosan CS and a sample CAC510 under degradation in active slurry solution as function of time

4. Conclusions

Chitosan-*g*-poly(acrylic acid) copolymers were successfully synthesized with the ceric ion. After 2 h of reaction at 70 ^{o}C, a gel product was obtained and a pseudo cross-linked structure was proposed. The swelling ratio of the copolymer has a maximum value in the buffer solution at pH 7. In addition, the swelling ratio of the copolymer increases with the feeding amount of acrylic acid monomer. For the sample with 40 g of AA monomer feed based on 5 g of chitosan, the swelling ration reached 32 at pH 7. The thermal degradation of the copolymer samples started at 200 ^{o}C and exhibited three stages of degradation. The degradation behavior of the copolymers was observed both in a lysozyme solution and an active slurry solution. Yet, the degradation rate was higher in the lysozyme solution and depended on the composition of the copolymers.

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